

EXPRESS MAIL CERTIFICATE

DATE 12/20/93 LABEL NO. B45293687X

I hereby certify that, on the date indicated above, I deposited this paper or fee with the U. S. Postal Service and that it was addressed for delivery to the Commissioner of Patents and Trademarks, Washington, DC 20311 by "Express Mail Post Office to Addressee" Service.

OKARASZI  
Name (Print)

G. K. Karaszi  
Signature

4444/08981

TRANSGENIC PRODUCTION OF ANTIBODIES IN MILK

Field of the Invention

This invention pertains to a method for the production of monoclonal antibodies in mammal's milk, specifically through the creation of transgenic animals that selectively express foreign antibody genes in mammary epithelial cells.

Background of the Invention

Immunoglobulins are heteropolymeric proteins that are normally synthesized, modified, assembled, and secreted from circulating B lymphocytes. Using recombinant DNA technology, it is possible to program cells other than B-lymphocytes to express immunoglobulin genes. The difficulties encountered in this effort stem from several factors: 1) Both heavy and light chains of immunoglobulins must be co-expressed at appropriate levels; 2) Nascent immunoglobulin polypeptides undergo a variety of co- and post-translational modifications that may not occur with sufficient fidelity or efficiency in heterologous cells; 3) Immunoglobulins require accessory chaperone proteins for their assembly; 4) The synthetic and secretory capacity of the cell may be inadequate to secrete large amounts of heterologous proteins; and 5) The secreted immunoglobulins may be unstable in the extracellular milieu of a foreign cell.

Because immunoglobulins have many therapeutic, diagnostic and industrial applications, there is a need in the art for expression systems in which these proteins can be reproducibly manufactured at a high level, in a functional configuration, and in

a form that allows them to be easily harvested and purified. The development of transgenic animal technology

has raised the possibility of using large animals as genetically programmed protein factories. P.C.T. application WO 90/04036

(published 4/19/90) discloses the use of transgenic technology for immunoglobulin expression. WO 92/03918 (3/19/92) and WO 93/12227

(6/24/93) teach the introduction of unrearranged immunoglobulin genes into the germline of transgenic animals. The use of intact

immunoglobulin genes (including their respective promoter regions)

will result in their expression in lymphocytes and secretion into

the bloodstream of the host animal; this necessitates a strategy

for suppressing the expression of the host's endogenous

immunoglobulins, and raises the problem of purifying the

immunoglobulins from serum, which contains many other proteins,

including proteolytic enzymes. Furthermore, if the transgenic

approach is chosen, heavy and light chain genes must both be

incorporated into the host genome, in a manner that enables their

concomitant expression.

Another option in creating transgenic animals is to link

the gene of interest to a heterologous transcriptional promoter

that only functions in a defined cell type within the host. In

this manner, tissue-specific expression of the transgene may be

programmed. U.S. Patent No. 4,873,316 (issued October 10, 1989)

discloses the production of recombinant tissue plasminogen

activator (TPA) in the milk of transgenic mice in which the TPA

gene is linked to the promoter of the milk protein casein. Other

proteins that have been expressed in a similar fashion include

cystic fibrosis transmembrane conductance regulator (DiTullio et

al., *Bio/Technology* 10:74, 1992), urokinase (Meade et al.,

*Bio/Technology* 8: 443, 1990), interleukin-2 (Buhler et al.,

*Bio/Technology* 8:140, 1990), and antihemophilic factor IX (Clark et

al., *Bio/Technology* 7:487, 1989). Notably, these proteins are all

simple single-chain polypeptides that do not require

multimerization or assembly prior to secretion.

090125004-012355  
8/14/94

It has now been found that when a transgenic mammal is created carrying paired immunoglobulin light and heavy chain genes under the control of the casein promoter, such an animal produces large amounts of assembled immunoglobulins which are secreted in its milk. Using the DNA constructs of the present invention, a surprisingly high efficiency of co-integration of heavy and light chain genes is observed. Using the method and constructs of the present invention, it is possible for the first time to program a mammary epithelial cell to produce and assemble complex tetrameric glycoproteins and secrete them in high quantities.

Accordingly, it is an object of the present invention to provide methods for the large-scale production of immunoglobulins in the milk of transgenic mammals.

Another object of the invention is to provide methods for the design of synthetic immunoglobulins that can be produced in large quantities in milk.

Yet another object of the invention is to provide methods for administering therapeutically beneficial antibodies to suckling young, by creating female mammals that excrete such antibodies into their milk.

A further object of the invention is a transgenic non-human mammal having germ and somatic cells with recombinant DNA sequences encoding immunoglobulin light and heavy chains, where said sequences are operatively linked at their 5' termini to a mammary specific promoter and at their 3' end to a sequence comprising a polyadenylation site.

A further object of the invention is a casein promoter cassette comprising in the 5' to 3' direction:

- a) 5' promoter sequences from the beta casein gene,
- b) an XhoI restriction site, and
- c) 3' untranslated sequences from the goat beta casein gene.

These and other objects of the present invention will be apparent to those of ordinary skill in the art in light of the present specification, drawings, and claims.

### Brief Description of the Drawings

Figure 1 is a schematic representation of the Bc62 plasmid, which contains a 13.9 kb Sal I fragment that comprises cDNA encoding immunoglobulin light chain, flanked on its 5' and 3' termini by goat beta casein sequences.

Figure 2 is a schematic representation of the Bc61 plasmid, which contains a 14.6 kb Sal I fragment that comprises cDNA encoding immunoglobulin heavy chain, flanked on its 5' and 3' termini by goat beta casein sequences.

Figure 3 depicts the immunoblot detection of human immunoglobulin heavy chain in the milk of transgenic mice that were created using the beta casein promoter-linked immunoglobulin genes shown in Figures 1 and 2.

Figure 4 depicts the immunoblot detection of human immunoglobulin light chain in the milk of transgenic mice that were created using the beta casein promoter-linked immunoglobulin genes shown in Figures 1 and 2.

### Summary of the Invention

In one aspect, this invention comprises a method for obtaining heterologous immunoglobulins from the milk of transgenic mammals. Another aspect of the present invention comprises the method for creating transgenic mammals by introducing into their germline immunoglobulin cDNA linked to a milk-specific promoter.

In another aspect, the present invention comprises transgenic mammals having germ cells and somatic cells having recombinant DNA sequences comprising immunoglobulin cDNA linked to a milk-specific promoter.

In still another aspect, the present invention comprises an isolated DNA comprising an expression cassette having 5' and 3' non-coding sequences derived from the goat beta casein gene linked via a unique restriction site that serves as a convenient cloning site for immunoglobulin coding sequences.

Detailed Description of the Invention

All patent applications, patents and literature cited in this specification are hereby incorporated by reference in their entirety. In the case of inconsistencies, the present disclosure will prevail.

The present invention pertains to a method for the production of monoclonal antibodies that are excreted into the milk of transgenic animals and the method for production of such animals. This is achieved by engineering DNA constructs in which DNA segments encoding specific paired immunoglobulin heavy and light chains are cloned downstream of a promoter sequence that is preferentially expressed in mammary epithelial cells. The recombinant DNAs containing the promoter-linked heavy and light chain genes are then coinjected into preimplantation embryos. The progeny are screened for the presence of both transgenes. Representative females from these lines are then milked, and the milk is analyzed for the presence of the monoclonal antibody. In order for the antibody to be present, both heavy and light chain genes must be expressed concurrently in the same cell. The antibodies may be purified from the milk, or the milk itself, comprising the immunoglobulins, may be used to deliver the antibodies to a recipient. This is discussed below.

The immunoglobulin genes useful in the present invention may be obtained from natural sources e.g. individual B cell clones or hybridomas derived therefrom. Alternately, they may comprise synthetic single-chain antibodies in which the light and heavy variable regions are expressed as part of a single polypeptide. Furthermore, recombinant antibody genes may be used that have been predictively altered by nucleotide substitutions that do or do not change the amino acid sequence, by addition or deletion of sequences, or by creation of hybrid genes in which different regions of the polypeptide are derived from different sources. Antibody genes by their nature are extremely diverse, and thus naturally tolerate a great deal of variation. It will be appreciated by those skilled in the art that the only limitation

for producing an antibody by the method of the present invention is that it must assemble into a functional configuration and be secreted in a stable form into the milk.

5 The transcriptional promoters useful in practicing the present invention are those promoters that are preferentially activated in mammary epithelial cells, including promoters that control the genes encoding milk proteins such as caseins, beta lactoglobulin (Clark et al., (1989) *Bio/Technology* 7: 487-492), whey acid protein (Gordon et al., (1987) *Bio/Technology* 5: 1183-1187), and lactalbumin (Soulier et al., (1992) *FEBS Letts.* 297: 13). Casein promoters may be derived from the alpha, beta, or kappa casein genes of any mammalian species; a preferred promoter is derived from the goat beta casein gene (DiTullio, (1992) *Bio/Technology* 10:74-77).

15 For use in the present invention, a unique XhoI restriction site is introduced at the 3' terminus of the promoter sequence to allow the routine insertion of immunoglobulin coding sequences. Preferably, the inserted immunoglobulin gene is flanked on its 3' side by cognate genomic sequences from a mammary-specific gene, to provide a polyadenylation site and transcript-stabilizing sequences. Transcription of the construct in vivo results in the production of a stable mRNA containing casein-derived 5' untranslated sequences upstream of the translational initiator codon of the immunoglobulin gene and 3' untranslated sequences downstream of the translational termination codon of the immunoglobulin gene. Finally, the entire cassette (i.e. promoter-immunoglobulin-3' region) is flanked by restriction sites that enable the promoter-cDNA cassette to be easily excised as a single fragment. This facilitates the removal of unwanted prokaryotic vector-derived DNA sequences prior to injection into fertilized eggs.

25 The promoter-linked immunoglobulin heavy and light chain DNAs are then introduced into the germ line of a mammal e.g. cow, sheep, goat, mouse, oxen, camel or pig. Mammals are defined herein as all animals, excluding humans, that have mammary glands and

produce milk. Mammalian species that produce milk in large amounts over long periods of time are preferred. Typically, the DNA is injected into the pronuclei of fertilized eggs, which are then implanted into the uterus of a recipient female and allowed to gestate. After birth, the putative transgenic animals are tested for the presence of the introduced DNA. This is easily achieved by Southern blot hybridization of DNA extracted from blood cells or other available tissue, using as a probe a segment of the injected gene that shows no cross hybridization with the DNA of the recipient species. Progeny that show evidence of at least one copy of both heavy and light-chain immunoglobulin genes are selected for further analysis.

Transgenic females may be tested for immunoglobulin secretion into milk, using any of the immunological techniques that are standard in the art (e.g. Western blot, radioimmunoassay, ELISA). The anti-immunoglobulin antibodies used in this analysis may be polyclonal or monoclonal antibodies that detect isolated heavy or light chains or others that react only with fully assembled (H2L2) immunoglobulins.

The recombinant immunoglobulins are also characterized with respect to their functionality, i.e. binding specificity and affinity for a particular antigen. This is achieved using immunological methods that are standard in the art, such as Scatchard analysis, binding to immobilized antigen, etc. The stability characteristics of an immunoglobulin in the milk of a given species are also assayed, by applying the above-described detection methods to milk that has been incubated for increasing times after recovery from the animal.

The immunoglobulins produced by the methods of the present invention may be purified from milk, using adsorption to immobilized Protein G, column chromatography, and other methods known to those of ordinary skill in the art of antibody purification.

The level of production of recombinant immunoglobulins in an individual transgenic mammal is primarily determined by the site

and manner of integration of the transgene after injection into the fertilized egg. Thus, transgenic progeny derived from different injected eggs may vary with respect to this parameter. The amount of recombinant immunoglobulin in milk is therefore monitored in representative progeny, and the highest-producing females are preferred.

Those skilled in the art will recognize that the methods of the present invention can be used to optimize the production of natural and synthetic immunoglobulins. The steps of creating a transgenic animal, testing for the presence of both heavy and light-chain genes, assaying the secretion of immunoglobulin into the milk of female progeny, and, finally, assessing the quality of the resulting antibodies, can be repeated sequentially, without undue experimentation, to establish preferred constructs for different applications.

According to the present invention, the nature of the recombinant immunoglobulins and their specific mode of use can vary. In one embodiment, the present invention encompasses high-level expression of antibodies that are harvested and purified from milk and used in purified form. High-level expression is defined herein as the production of about 1 mg/ml of protein. In another embodiment, antibodies are engineered that provide protection to humans against infectious diseases; therapeutic administration is then achieved by drinking the milk. In a still further embodiment, lactating animals are engineered to produce antibodies specifically beneficial to their offspring, which acquire them through suckling. In a still further embodiment, animals produce an antibody that protects the lactating mammal itself against breast pathogens e.g. bacteria that produce mastitis.

The unexpectedly high-volume expression of immunoglobulins using the method and constructs of the present invention also allows the use of such immunoglobulins in pharmaceutical and chemical settings. By way of non-limiting example the method of the present invention can be used to produce high levels of tetrameric antibodies directed against various



pathogens (e.g. *E. coli*, *Salmonella*, hepatitis B virus), biologically active peptides (e.g. erythropoietin, tissue plasminogen activator, gamma interferon) and for use in chemical reactions directed against various enzymes. Monoclonal antibodies that bind to the transition state of a chemical reaction can be used in industrial-scale production. Furthermore, monoclonal antibodies are often immobilized on columns for use in the purification of biopharmaceuticals; in such cases, production of the antibodies represents a significant fraction of the cost of purification. The methods of the present invention facilitate the production of high-volume, low cost antibody stocks for use in these types of applications.

The present invention is further described in the following working examples, which are intended to illustrate the invention without limiting its scope.

Example 1: Construction of a Milk-Specific Promoter Cassette

The present invention encompasses a recipient vector into which many different immunoglobulin genes can be interchangeably inserted. The vector contains 5' milk-specific promoter sequences and 3' untranslated genomic sequences that flank an XhoI cloning site. This cloning is unique because it is the only one present in the vector. Preferably, the entire expression cassette should be flanked by restriction sites that allow the easy excision of the promoter-linked immunoglobulin gene.

In this Example, the promoter and 3' genomic sequences were derived from the goat beta casein gene. The gene was cloned and characterized as described by Roberts et al., 1992, *Gene* 121:255-262, which is hereby incorporated by reference.

The expression cassette, prior to insertion of immunoglobulin genes, consists of 6.2 kb upstream of the translational start of the beta casein coding sequence and 7.1 kb of genomic sequence downstream of the translational stop of the beta casein gene. The TaqI site just upstream of the translational start codon was changed to an XhoI site. This unique XhoI cloning

site is at the junction of the upstream and downstream sequences. It is this XhoI site, included in the sequence CGCGGATCCTCGAGGACC, into which recombinant immunoglobulin genes are inserted. (D. Tullio, (1992) *Bio/Technology* 10:74-77).

5 The 3' beta casein region begins at the PpuMI site found  
SUB in Exon 7 and continues for 7.1 kb downstream. Included in this  
DZ sequence are the remaining 18 bp of Exon 7, and all of Exon 8 and  
Exon 9. These encode the 3' untranslated regions of the goat beta  
casein gene, and terminate with the sequence:  
10 TAAGGTCCACAGACCGAGACCCACTCACTAGGCAACTGGTCCGTCCAGCTGTTAAGTGA.

To engineer restriction sites flanking the casein  
cassette, the goat beta casein control sequences were first cloned  
into the SuperCos1 vector (#251301, Stratagene, La Jolla, CA) with  
flanking NotI and SalI sites. This plasmid was then modified by  
changing the NotI site to a SalI site. This created a 13.3 kb  
SalI fragment containing the beta casein expression cassette within  
the gbc163 vector.

#### Example 2: Construction of Promoter-linked Monoclonal Antibody Genes

20 In this Example, the genes encoding a human monoclonal  
antibody directed against a colon cancer cell-surface marker were  
linked to the casein promoter. cDNAs encoding the light and heavy  
chains of this antibody were cloned from an antibody-secreting  
hybridoma cell line into a pUC19-derived vector. The light and  
heavy chain cDNAs were present on HindIII/EcoRI fragments of 702 bp  
25 and 1416 bp, respectively.

To adapt the genes for insertion into the casein promoter  
cassette, XhoI restriction sites were engineered at both ends of  
each DNA segment as detailed below. In the same step, the region  
upstream of the immunoglobulin translation initiation codon was  
30 modified so that it contained sequences similar to those in the  
analogous region of the beta casein gene.

Light chain gene: The pUC19 plasmid containing the light  
chain cDNA insert was digested with HindIII, blunt-ended by  
treatment with the Klenow fragment of DNA Polymerase I, and ligated

to an oligonucleotide containing an XhoI recognition sequence (#1030, New England Biolabs, Beverly, MA).

5UB  
5 D3  
The region immediately upstream of the initiating ATG was then mutagenized using an oligonucleotide with the following sequence: 5' AGT GAA TTC ATG CTC GAG AGC CAT GGC CTG GATC 3'. Digestion of the final plasmid with XhoI produced the modified light chain cDNA that was flanked by XhoI cohesive ends.

0 The light chain cDNA was then inserted into the unique XhoI cloning site of the gbc163 expression vector described in Example 1, yielding plasmid Bc62 (Figure 1).

5  
10  
15  
20  
25  
30  
35  
40  
45  
50  
55  
60  
65  
70  
75  
80  
85  
90  
95  
100  
105  
110  
115  
120  
125  
130  
135  
140  
145  
150  
155  
160  
165  
170  
175  
180  
185  
190  
195  
200  
205  
210  
215  
220  
225  
230  
235  
240  
245  
250  
255  
260  
265  
270  
275  
280  
285  
290  
295  
300  
305  
310  
315  
320  
325  
330  
335  
340  
345  
350  
355  
360  
365  
370  
375  
380  
385  
390  
395  
400  
405  
410  
415  
420  
425  
430  
435  
440  
445  
450  
455  
460  
465  
470  
475  
480  
485  
490  
495  
500  
505  
510  
515  
520  
525  
530  
535  
540  
545  
550  
555  
560  
565  
570  
575  
580  
585  
590  
595  
600  
605  
610  
615  
620  
625  
630  
635  
640  
645  
650  
655  
660  
665  
670  
675  
680  
685  
690  
695  
700  
705  
710  
715  
720  
725  
730  
735  
740  
745  
750  
755  
760  
765  
770  
775  
780  
785  
790  
795  
800  
805  
810  
815  
820  
825  
830  
835  
840  
845  
850  
855  
860  
865  
870  
875  
880  
885  
890  
895  
900  
905  
910  
915  
920  
925  
930  
935  
940  
945  
950  
955  
960  
965  
970  
975  
980  
985  
990  
995  
1000  
1005  
1010  
1015  
1020  
1025  
1030  
1035  
1040  
1045  
1050  
1055  
1060  
1065  
1070  
1075  
1080  
1085  
1090  
1095  
1100  
1105  
1110  
1115  
1120  
1125  
1130  
1135  
1140  
1145  
1150  
1155  
1160  
1165  
1170  
1175  
1180  
1185  
1190  
1195  
1200  
1205  
1210  
1215  
1220  
1225  
1230  
1235  
1240  
1245  
1250  
1255  
1260  
1265  
1270  
1275  
1280  
1285  
1290  
1295  
1300  
1305  
1310  
1315  
1320  
1325  
1330  
1335  
1340  
1345  
1350  
1355  
1360  
1365  
1370  
1375  
1380  
1385  
1390  
1395  
1400  
1405  
1410  
1415  
1420  
1425  
1430  
1435  
1440  
1445  
1450  
1455  
1460  
1465  
1470  
1475  
1480  
1485  
1490  
1495  
1500  
1505  
1510  
1515  
1520  
1525  
1530  
1535  
1540  
1545  
1550  
1555  
1560  
1565  
1570  
1575  
1580  
1585  
1590  
1595  
1600  
1605  
1610  
1615  
1620  
1625  
1630  
1635  
1640  
1645  
1650  
1655  
1660  
1665  
1670  
1675  
1680  
1685  
1690  
1695  
1700  
1705  
1710  
1715  
1720  
1725  
1730  
1735  
1740  
1745  
1750  
1755  
1760  
1765  
1770  
1775  
1780  
1785  
1790  
1795  
1800  
1805  
1810  
1815  
1820  
1825  
1830  
1835  
1840  
1845  
1850  
1855  
1860  
1865  
1870  
1875  
1880  
1885  
1890  
1895  
1900  
1905  
1910  
1915  
1920  
1925  
1930  
1935  
1940  
1945  
1950  
1955  
1960  
1965  
1970  
1975  
1980  
1985  
1990  
1995  
2000  
2005  
2010  
2015  
2020  
2025  
2030  
2035  
2040  
2045  
2050  
2055  
2060  
2065  
2070  
2075  
2080  
2085  
2090  
2095  
2100  
2105  
2110  
2115  
2120  
2125  
2130  
2135  
2140  
2145  
2150  
2155  
2160  
2165  
2170  
2175  
2180  
2185  
2190  
2195  
2200  
2205  
2210  
2215  
2220  
2225  
2230  
2235  
2240  
2245  
2250  
2255  
2260  
2265  
2270  
2275  
2280  
2285  
2290  
2295  
2300  
2305  
2310  
2315  
2320  
2325  
2330  
2335  
2340  
2345  
2350  
2355  
2360  
2365  
2370  
2375  
2380  
2385  
2390  
2395  
2400  
2405  
2410  
2415  
2420  
2425  
2430  
2435  
2440  
2445  
2450  
2455  
2460  
2465  
2470  
2475  
2480  
2485  
2490  
2495  
2500  
2505  
2510  
2515  
2520  
2525  
2530  
2535  
2540  
2545  
2550  
2555  
2560  
2565  
2570  
2575  
2580  
2585  
2590  
2595  
2600  
2605  
2610  
2615  
2620  
2625  
2630  
2635  
2640  
2645  
2650  
2655  
2660  
2665  
2670  
2675  
2680  
2685  
2690  
2695  
2700  
2705  
2710  
2715  
2720  
2725  
2730  
2735  
2740  
2745  
2750  
2755  
2760  
2765  
2770  
2775  
2780  
2785  
2790  
2795  
2800  
2805  
2810  
2815  
2820  
2825  
2830  
2835  
2840  
2845  
2850  
2855  
2860  
2865  
2870  
2875  
2880  
2885  
2890  
2895  
2900  
2905  
2910  
2915  
2920  
2925  
2930  
2935  
2940  
2945  
2950  
2955  
2960  
2965  
2970  
2975  
2980  
2985  
2990  
2995  
3000  
3005  
3010  
3015  
3020  
3025  
3030  
3035  
3040  
3045  
3050  
3055  
3060  
3065  
3070  
3075  
3080  
3085  
3090  
3095  
3100  
3105  
3110  
3115  
3120  
3125  
3130  
3135  
3140  
3145  
3150  
3155  
3160  
3165  
3170  
3175  
3180  
3185  
3190  
3195  
3200  
3205  
3210  
3215  
3220  
3225  
3230  
3235  
3240  
3245  
3250  
3255  
3260  
3265  
3270  
3275  
3280  
3285  
3290  
3295  
3300  
3305  
3310  
3315  
3320  
3325  
3330  
3335  
3340  
3345  
3350  
3355  
3360  
3365  
3370  
3375  
3380  
3385  
3390  
3395  
3400  
3405  
3410  
3415  
3420  
3425  
3430  
3435  
3440  
3445  
3450  
3455  
3460  
3465  
3470  
3475  
3480  
3485  
3490  
3495  
3500  
3505  
3510  
3515  
3520  
3525  
3530  
3535  
3540  
3545  
3550  
3555  
3560  
3565  
3570  
3575  
3580  
3585  
3590  
3595  
3600  
3605  
3610  
3615  
3620  
3625  
3630  
3635  
3640  
3645  
3650  
3655  
3660  
3665  
3670  
3675  
3680  
3685  
3690  
3695  
3700  
3705  
3710  
3715  
3720  
3725  
3730  
3735  
3740  
3745  
3750  
3755  
3760  
3765  
3770  
3775  
3780  
3785  
3790  
3795  
3800  
3805  
3810  
3815  
3820  
3825  
3830  
3835  
3840  
3845  
3850  
3855  
3860  
3865  
3870  
3875  
3880  
3885  
3890  
3895  
3900  
3905  
3910  
3915  
3920  
3925  
3930  
3935  
3940  
3945  
3950  
3955  
3960  
3965  
3970  
3975  
3980  
3985  
3990  
3995  
4000  
4005  
4010  
4015  
4020  
4025  
4030  
4035  
4040  
4045  
4050  
4055  
4060  
4065  
4070  
4075  
4080  
4085  
4090  
4095  
4100  
4105  
4110  
4115  
4120  
4125  
4130  
4135  
4140  
4145  
4150  
4155  
4160  
4165  
4170  
4175  
4180  
4185  
4190  
4195  
4200  
4205  
4210  
4215  
4220  
4225  
4230  
4235  
4240  
4245  
4250  
4255  
4260  
4265  
4270  
4275  
4280  
4285  
4290  
4295  
4300  
4305  
4310  
4315  
4320  
4325  
4330  
4335  
4340  
4345  
4350  
4355  
4360  
4365  
4370  
4375  
4380  
4385  
4390  
4395  
4400  
4405  
4410  
4415  
4420  
4425  
4430  
4435  
4440  
4445  
4450  
4455  
4460  
4465  
4470  
4475  
4480  
4485  
4490  
4495  
4500  
4505  
4510  
4515  
4520  
4525  
4530  
4535  
4540  
4545  
4550  
4555  
4560  
4565  
4570  
4575  
4580  
4585  
4590  
4595  
4600  
4605  
4610  
4615  
4620  
4625  
4630  
4635  
4640  
4645  
4650  
4655  
4660  
4665  
4670  
4675  
4680  
4685  
4690  
4695  
4700  
4705  
4710  
4715  
4720  
4725  
4730  
4735  
4740  
4745  
4750  
4755  
4760  
4765  
4770  
4775  
4780  
4785  
4790  
4795  
4800  
4805  
4810  
4815  
4820  
4825  
4830  
4835  
4840  
4845  
4850  
4855  
4860  
4865  
4870  
4875  
4880  
4885  
4890  
4895  
4900  
4905  
4910  
4915  
4920  
4925  
4930  
4935  
4940  
4945  
4950  
4955  
4960  
4965  
4970  
4975  
4980  
4985  
4990  
4995  
5000  
5005  
5010  
5015  
5020  
5025  
5030  
5035  
5040  
5045  
5050  
5055  
5060  
5065  
5070  
5075  
5080  
5085  
5090  
5095  
5100  
5105  
5110  
5115  
5120  
5125  
5130  
5135  
5140  
5145  
5150  
5155  
5160  
5165  
5170  
5175  
5180  
5185  
5190  
5195  
5200  
5205  
5210  
5215  
5220  
5225  
5230  
5235  
5240  
5245  
5250  
5255  
5260  
5265  
5270  
5275  
5280  
5285  
5290  
5295  
5300  
5305  
5310  
5315  
5320  
5325  
5330  
5335  
5340  
5345  
5350  
5355  
5360  
5365  
5370  
5375  
5380  
5385  
5390  
5395  
5400  
5405  
5410  
5415  
5420  
5425  
5430  
5435  
5440  
5445  
5450  
5455  
5460  
5465  
5470  
5475  
5480  
5485  
5490  
5495  
5500  
5505  
5510  
5515  
5520  
5525  
5530  
5535  
5540  
5545  
5550  
5555  
5560  
5565  
5570  
5575  
5580  
5585  
5590  
5595  
5600  
5605  
5610  
5615  
5620  
5625  
5630  
5635  
5640  
5645  
5650  
5655  
5660  
5665  
5670  
5675  
5680  
5685  
5690  
5695  
5700  
5705  
5710  
5715  
5720  
5725  
5730  
5735  
5740  
5745  
5750  
5755  
5760  
5765  
5770  
5775  
5780  
5785  
5790  
5795  
5800  
5805  
5810  
5815  
5820  
5825  
5830  
5835  
5840  
5845  
5850  
5855  
5860  
5865  
5870  
5875  
5880  
5885  
5890  
5895  
5900  
5905  
5910  
5915  
5920  
5925  
5930  
5935  
5940  
5945  
5950  
5955  
5960  
5965  
5970  
5975  
5980  
5985  
5990  
5995  
6000  
6005  
6010  
6015  
6020  
6025  
6030  
6035  
6040  
6045  
6050  
6055  
6060  
6065  
6070  
6075  
6080  
6085  
6090  
6095  
6100  
6105  
6110  
6115  
6120  
6125  
6130  
6135  
6140  
6145  
6150  
6155  
6160  
6165  
6170  
6175  
6180  
6185  
6190  
6195  
6200  
6205  
6210  
6215  
6220  
6225  
6230  
6235  
6240  
6245  
6250  
6255  
6260  
6265  
6270  
6275  
6280  
6285  
6290  
6295  
6300  
6305  
6310  
6315  
6320  
6325  
6330  
6335  
6340  
6345  
6350  
6355  
6360  
6365  
6370  
6375  
6380  
6385  
6390  
6395  
6400  
6405  
6410  
6415  
6420  
6425  
6430  
6435  
6440  
6445  
6450  
6455  
6460  
6465  
6470  
6475  
6480  
6485  
6490  
6495  
6500  
6505  
6510  
6515  
6520  
6525  
6530  
6535  
6540  
6545  
6550  
6555  
6560  
6565  
6570  
6575  
6580  
6585  
6590  
6595  
6600  
6605  
6610  
6615  
6620  
6625  
6630  
6635  
6640  
6645  
6650  
6655  
6660  
6665  
6670  
6675  
6680  
6685  
6690  
6695  
6700  
6705  
6710  
6715  
6720  
6725  
6730  
6735  
6740  
6745  
6750  
6755  
6760  
6765  
6770  
6775  
6780  
6785  
6790  
6795  
6800  
6805  
6810  
6815  
6820  
6825  
6830  
6835  
6840  
6845  
6850  
6855  
6860  
6865  
6870  
6875  
6880  
6885  
6890  
6895  
6900  
6905  
6910  
6915  
6920  
6925  
6930  
6935  
6940  
6945  
6950  
6955  
6960  
6965  
6970  
6975  
6980  
6985  
6990  
6995  
7000  
7005  
7010  
7015  
7020  
7025  
7030  
7035  
7040  
7045  
7050  
7055  
7060  
7065  
7070  
7075  
7080  
7085  
7090  
7095  
7100  
7105  
7110  
7115  
7120  
7125  
7130  
7135  
7140  
7145  
7150  
7155  
7160  
7165  
7170  
7175  
7180  
7185  
7190  
7195  
7200  
7205  
7210  
7215  
7220  
7225  
7230  
7235  
7240  
7245  
7250  
7255  
7260  
7265  
7270  
7275  
7280  
7285  
7290  
7295  
7300  
7305  
7310  
7315  
7320  
7325  
7330  
7335  
7340  
7345  
7350  
7355  
7360  
7365  
7370  
7375  
7380  
7385  
7390  
7395  
7400  
7405  
7410  
7415  
7420  
7425  
7430  
7435  
7440  
7445  
7450  
7455  
7460  
7465  
7470  
7475  
7480  
7485  
7490  
7495  
7500  
7505  
7510  
7515  
7520  
7525  
7530  
7535  
7540  
7545  
7550  
7555  
7560  
7565  
7570  
7575  
7580  
7585  
7590  
7595  
7600  
7605  
7610  
7615  
7620  
7625  
7630  
7635  
7640  
7645  
7650  
7655  
7660  
7665  
7670  
7675  
7680  
7685  
7690  
7695  
7700  
7705  
7710  
7715  
7720  
7725  
7730  
7735  
7740  
7745  
7750  
7755  
7760  
7765  
7770  
7775  
7780  
7785  
7790  
7795  
7800  
7805  
7810  
7815  
7820  
7825  
7830  
7835  
7840  
7845  
7850  
7855  
7860  
7865  
7870  
7875  
7880  
7885  
7890  
7895  
7900  
7905  
7910  
7915  
7920  
7925  
7930  
7935  
7940  
7945  
7950  
7955  
7960  
7965  
7970  
7975  
7980  
7985  
7990  
7995  
8000  
8005  
8010  
8015  
8020  
8025  
8030  
8035  
8040  
8045  
8050  
8055  
8060  
8065  
8070  
8075  
8080  
8085  
8090  
8095  
8100  
8105  
8110  
8115  
8120  
8125  
8130  
8135  
8140  
8145  
8150  
8155  
8160  
8165  
8170  
8175  
8180  
8185  
8190  
8195  
8200  
8205  
8210  
8215  
8220  
8225  
8230  
8235  
8240  
8245  
8250  
8255  
8260  
8265  
8270  
8275  
8280  
8285  
8290  
8295  
8300  
8305  
8310  
8315  
8320  
8325  
8330  
8335  
8340  
8345  
8350  
8355  
8360  
8365  
8370  
8375  
8380  
8385  
8390  
8395  
8400  
8405  
8410  
8415  
8420  
8425  
8430  
8435  
8440  
8445  
8450  
8455  
8460  
8465  
8470  
8475  
8480  
8485  
8490  
8495  
8500  
8505  
8510  
8515  
8520  
8525  
8530  
8535  
8540  
8545  
8550  
8555  
8560  
8565  
8570  
8575  
8580  
8585  
8590  
8595  
8600  
8605  
8610  
8615  
8620  
8625  
8630  
8635  
8640  
8645  
8650  
8655  
8660  
8665  
8670  
8675  
8680  
8685  
8690  
8695  
8700  
8705  
8710  
8715  
8720  
8725  
8730  
8735  
8740  
8745  
8750  
8755  
8760  
8765  
8770  
8775  
8780  
8785  
8790  
8795  
8800  
8805  
8810  
8815  
8820  
8825  
8830  
8835  
8840  
8845  
8850  
8855  
8860  
8865  
8870  
8875  
8880  
8885  
8890  
8895  
8900  
8905  
8910  
8915  
8920  
8925  
8930  
8935  
8940  
8945  
8950  
8955  
8960  
8965  
8970  
8975  
8980  
8985  
8990  
8995  
9000  
9005  
9010  
9015  
9020  
9025  
9030  
9035  
9040  
9045  
9050  
9055  
9060  
9065  
9070  
9075  
9080  
9085  
9090  
9095  
9100  
9105  
9110  
9115  
9120  
9125  
9130  
9135  
9140  
9145  
9150  
9155  
9160  
9165  
9170  
9175  
9180  
9185  
9190  
9195  
9200  
9205  
9210  
9215  
9220  
9225  
9230  
9235  
9240  
9245  
9250  
9255  
9260  
9265  
9270  
9275  
9280  
9285  
9290  
9295  
9300  
9305  
9310  
9315  
9320  
9325  
9330  
9335  
9340  
9345  
9350  
9355  
9360  
9365  
9370  
9375  
9380  
9385  
9390  
9395  
9400  
9405  
9410  
9415  
9420  
9425  
9430  
9435  
9440  
9445  
9450  
9455  
9460  
9465  
9470  
9475  
9480  
9485  
9490  
9495  
9500  
9505  
9510  
9515  
9520  
9525  
9530  
9535  
9540  
9545  
9550  
9555  
9560  
9565  
9570  
9575  
9580  
9585  
9590  
9595  
9600  
9605  
9610  
9615  
9620  
9625  
9630  
9635  
9640  
9645  
9650  
9655  
9660  
9665  
9670  
9675  
9680  
9685  
9690  
9695  
9700  
9705  
9710  
9715  
9720  
9725  
9730  
9735  
9740  
9745  
9750  
9755  
9760  
9765  
9770  
9775  
9780  
9785  
9790  
9795  
9800  
9805  
9810  
9815  
9820  
9825  
9830  
9835  
9840  
9845  
9850  
9855  
9860  
9865  
9870  
9875  
9880  
9885  
9890  
9895  
9900  
9905  
9910  
9915  
9920  
9925  
9930  
9935  
9940  
9945  
9950  
9955  
9960  
9965  
9970  
9975  
9980  
9985  
9990  
9995  
10000  
10005  
10010  
10015  
10020  
10025  
10030  
10035  
10040  
10045  
10050  
10055  
10060  
10065  
10070  
10075  
10080  
10085  
10090  
10095  
10100  
10105  
10110  
10115  
10120  
10125  
10130  
10135  
10140  
10145  
10150  
10155  
10160  
10165  
10170  
10175  
10180  
10185  
10190  
10195  
10200

antibody gene sequences. DNA was extracted from tail biopsy material and probed using Southern blot analysis. The probes used in the hybridization were the original cDNAs encoding the heavy and light chains. As seen in Table 1, most of the first generation transgenic progeny had incorporated both transgenes.

Table 1  
Summary of Bc61 - Bc62 Mice

Founder	Sex	Bc61	Bc62	Expression
1-2	M	Pos.	Pos.	
1-3	M	Pos.	Pos.	light chain only
1-9	M	Pos.	Pos.	
1-15	F	Neg.	Pos.	Low level lambda chain
1-16	F	Pos.	Neg.	
1-19	F	Pos.	Pos.	N.D.
1-23	F	Pos.	Pos.	1-3 mg/ml
1-24	F	Pos.	Pos.	low level
1-25	M	Pos.	Neg.	
1-39	M	Pos.	Pos.	
1-13	F	Pos.	Pos.	N.D.
1-56	F	Pos.	Pos.	N.D.
1-64	M	Pos.	Pos.	
2-76	F	Pos.	Pos.	1-3 mg/ml
2-82	F	Pos.	Pos.	1-3 mg/ml
1-72	M	Pos.	Pos.	
2-92	F	Pos.	Pos.	0.2 - 0.5 mg/ml
2-95	F	Pos.	Pos.	0.2 - 0.5 mg/ml

N.D. = not detected

Example 4: Analysis of Recombinant Immunoglobulins in Milk

Samples of milk from the transgenic mice obtained as described in Example 3 were analyzed for the presence of the

heterologous immunoglobulin by Western blot. The heavy chain of the antibody was detected using a horseradish peroxide-linked polyclonal antibody directed against human gamma heavy chain (Antibody #62-8420, Zymed, South San Francisco, CA.) as shown in Figure 3. The light chain was detected using antibodies to the human lambda light chain, (Antibody #05-4120, Zymed, South San Francisco, CA) shown in Figure 4. In these Figures, it can be seen that immunoreactive heavy and light chains can be detected in the milk of several animals, but not in the negative control animal CD-

1. Human immunoglobulin can be detected in milk from founder 1-23 and from the progeny of the 1-76 and 1-72 founders. These animals are the second-generation females, 2-76, 2-82, 2-92, and 2-95. The levels of expression range between 0.2 mg/ml to over 1 mg/ml (Table 1).

0001004-01399